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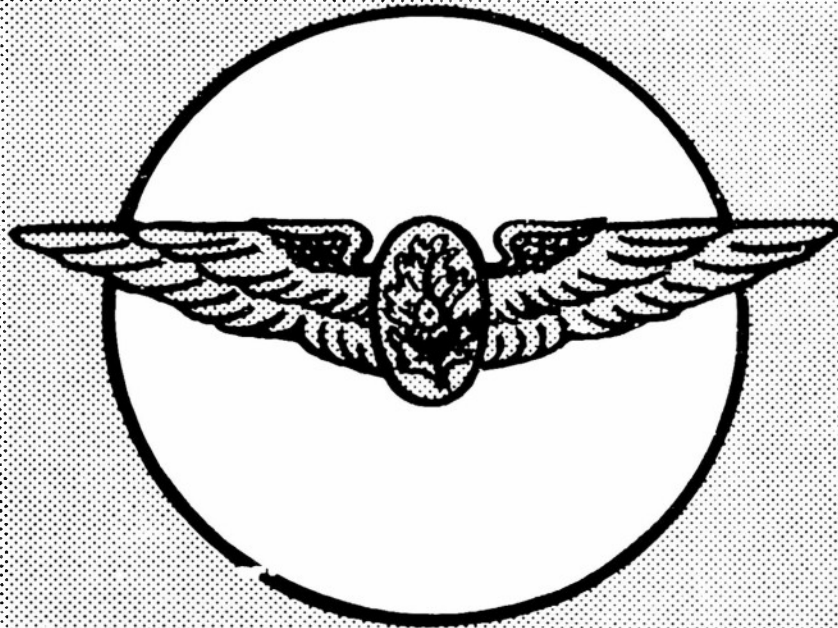
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ANALYSIS OF RENAL CALCULI BY  
INFRARED SPECTROSCOPY

REPORT NUMBER NM 001 062.01.01



# RESEARCH REPORT

OF THE

**U. S. NAVAL SCHOOL OF AVIATION MEDICINE**

NAVAL AIR STATION

PENSACOLA FLORIDA

U. S. NAVAL SCHOOL OF AVIATION MEDICINE  
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JOINT PROJECT REPORT NO. 1

The Butt-Douglas Research Foundation under Contract Nonr 926-00  
Office of Naval Research, Project Designation NR 103-011  
U. S. Naval School of Aviation Medicine

The Bureau of Medicine and Surgery  
Project No. NM 001 082.01.01

ANALYSIS OF RENAL CALCULI BY INFRARED SPECTROSCOPY

Report by

Dietrich E. Beischer, Ph.D.

Approved by

Captain Ashton Graybiel, MC USN  
Director of Research  
U. S. Naval School of Aviation Medicine

Released by

Captain James L. Holland, MC USN  
Commanding Officer

1 July 1954

## SUMMARY

The infrared spectra of mulled renal calculi are useful in the qualitative identification of the components of stones. An identification template with the most common components of renal calculi is helpful. Permanent analytical records may be obtained from small samples (5 mg) in a short time.

## INTRODUCTION

The importance of a knowledge of the chemical composition of renal calculi is accepted generally(6). Reliable analytical information is fundamental for a study of the etiology of stone formation and is absolutely necessary for planning medical regimens. While the classical chemical analysis is confronted with a number of difficulties in establishing the composition of stones, two physical methods have recently been found to be most helpful in this effort: the optical and the X-ray methods, discussed by Prien and Frondel(7).

The widespread acceptance of the infrared spectroscopy as an analytical research tool in many industrial laboratories suggested its use as a new method for the analysis of renal calculi. Infrared spectroscopy had already been used successfully in a similar application, the determination of mineral constituents of rocks(2,3,4). The speed and accuracy combined with the convenience of the commercially available infrared spectrometers strongly recommend their application in the clinical laboratory.

## PROCEDURE FOR INFRARED ANALYSIS

A double beam recording infrared spectrophotometer from the Perkin-Elmer Corporation, Norwalk, Connecticut, was used in these studies. The infrared radiation emitted from a Nernst glower leaves the glower housing at the right side of the instrument in the form of two parallel beams of equal intensity. One beam passes through the sample and the other beam serves to compensate the absorption of infrared radiation by water vapor and carbon dioxide on the way from the source to the monochromator which is housed under the recording drum. The infrared spectrum from 2 to 15 microns wave-lengths can be recorded with a sodium chloride prism in the monochromator. The pen system of the recorder indicates directly the transmittance in percent transmission of the sample. Usually, the spectra were scanned from 2 to 15 microns in 20 minutes. This time can be shortened if necessary.

The samples were prepared by the mulling technique. A small piece of a calculus or a sample of a reference substance was ground with the mulling agent, usually mineral oil (Nujol), to form a paste with a very fine particle size. This paste was squeezed between two windows of the microcell

to a desired thickness. This thickness cannot be controlled accurately, but with some practice adequate sample films can be produced for qualitative and semi-quantitative analysis. Less than 5 mg solid substance are adequate to cover the exposed area. The sample can be recovered after the test if necessary.

The renal calculi examined by this method were of recent origin and not museum specimens. They were chemically analyzed by the usual methods. In addition infrared spectra of characteristic components of renal calculi served as references. Some of these reference materials, such as calcium oxalate monohydrate, dibasic calcium phosphate dihydrate, tertiary calcium phosphate, uric acid and cystine were used in the form of commercially available products of highest purity. Other materials were synthesized. Magnesium ammonium phosphate was prepared by the method usually applied in analytical chemistry to determine magnesium or phosphorous. Hydroxy apatite was gained from a neutral calcium chloride solution mixed with ammonium phosphate in the presence of ammonium chloride and ammonia. The precipitate was analyzed after a week's crystallization time. For the preparation of carbonate apatite an addition of ammonium carbonate was made to the substances forming hydroxy apatite. The monohydrate of calcium oxalate was prepared following Schmidt (9) as precipitate from a neutral solution of calcium chloride by an excess of ammonium oxalate.

### RESULTS

The infrared spectra of four renal calculi are reproduced (fig. 1). They represent a selection of a greater number of similar spectra of calculi which had been analyzed previously by chemical methods. Such a collection of infrared spectra of analyzed stones served as reference in the identification of unknown stones. An identification template (fig. 2) in which the infrared spectra of the major components of renal calculi are assembled, was of further help in the identification of the components which are usually found in renal calculi. The spectra in this template were gained from commercially available pure chemicals or products synthesized as described above. These spectra were verified by comparison with published data (apatite(4), calcium hydrogen phosphate(5), phosphorus compounds(1), calcium oxalate monohydrate(3), uric acid(8), cystine(8)).

The chemical analysis of the calcium oxalate stone (fig. 1) indicated a small but definite amount of phosphorous which corresponded to an apatite concentration of below 1%. The presence of apatite is noticeable by a slight dip of the spectral curve around 9.5 microns. The wide absorption band of phosphates between 9 and 10 microns limits the minimal amount of apatite which can be recognized in a mixture to about one percent.



According to chemical analysis the calcium oxalate-calcium phosphate stone (fig. 1) was a mixture of equal parts of calcium oxalate and calcium phosphate in the form of hydroxy apatite. The intensities of the absorption maxima characteristic for the two components vary in binary stones according to proportion of the components in the mixture. With some experience semi-quantitative conclusions about the composition of the binary mixtures can be reached. Further information about the quantitative evaluation of the infrared spectra of stones will be discussed later.

The chemical analysis of the third stone (fig. 1) gave a composition of 85% magnesium ammonium phosphate and about 10% calcium oxalate with a rest of 5% unidentified. The maximum of the absorption of magnesium ammonium phosphate is slightly shifted to longer wavelength compared with the maximum found in the spectra of apatite. This shift is sufficient to identify the presence of magnesium ammonium phosphate. The close proximity of the broad maxima of absorption of the two phosphates makes the detection of a small amount of one component in the presence of a large amount of the other component difficult. However, 5 percent of apatite could be detected in a magnesium ammonium phosphate stone.

The stone mixed of cystine and uric acid (fig. 1) represents an example of the convenient identification of calculi with this kind of components. The chemical analysis of this stone had demonstrated the presence of cystine and uric acid in about equal amounts.

#### DISCUSSION

The empirical qualitative identification of urinary calculi by infrared analysis follows the general principle of comparing their spectra with the spectra of known compounds. Certain limitations of this procedure as far as they concern the analysis of urinary calculi have to be discussed.

A separation of the mono- and di-hydrate of calcium oxalate which is easily accomplished by optical analysis in the visible light, was not yet possible by infrared analysis. The synthesized pure hydrates, including the tri-hydrate, showed the same infrared absorption spectra. The absorption bands observed in the spectrum from 2 to 15 microns are connected with vibrations inside the oxalate anion and the water molecules. Vibrations between the calcium ion and the oxalate ion or the water molecules and the two ions which could be used for an identification of the different hydrates are located in the far infrared, inaccessible to the instrument used in this study.

The phosphate spectra of urinary calculi also need some discussion. The maximum of the absorption at about 10 microns is connected with vibrations inside the  $\text{PO}_4$ -group. These vibrations are not influenced noticeably by the presence of an OH-group in the slightly basic calcium phosphate with apatite lattice. Synthetic hydroxy apatite and commercial tertiary calcium phosphate showed practically the same infrared absorption. The influence of the OH-group on the rest of the anion in the hydroxy apatite may be concealed by the wide extension of the maximum at 10 microns. Since it was established by X-ray studies(2) that the  $\text{PO}_4$ -group in calcium salts of renal calculi is present in the form of a basic calcium phosphate, an absorption maximum at 9.65 microns is considered an indication of the presence of hydroxy apatite.

Figure 1 includes the infrared absorption spectrum of synthesized carbonate apatite. The broad maximum at 7.1 microns and weaker maxima at 11.4 and 13.9 are characteristic for the presence of calcium carbonate, very likely bound in the stone in the form of apatite. The carbonate apatite spectrum may be considered as a superimposition of the absorption spectra of tertiary calcium phosphate and calcium carbonate. No mutual influence of the  $\text{PO}_4$  and  $\text{CO}_3$  -ion in the apatite molecule could be observed in the infrared spectra.

The influence of the cations on the position of the  $\text{PO}_4$ -absorption maximum is well observable. The dislocation of the  $\text{PO}_4$ -maximum from 9.65 microns for the calcium compound to 10 microns in the magnesium ammonium phosphate was already mentioned as a means to distinguish between these two compounds. Some weaker absorption maxima in the infrared spectrum of magnesium ammonium phosphate can be associated with vibrations inside the  $\text{NH}_4$ -group. They may serve as further means of identification of magnesium ammonium phosphate.

The spectrum of calcium hydrogen phosphate dihydrate (secondary calcium phosphate) readily distinguishes this compound from the tertiary phosphate. The template (fig. 2) shows a broad absorption maximum with several sub-maxima. Since  $\text{CaHPO}_4$  is a rather uncommon phosphate in urinary calculi no stones with this compound were available for analysis.

The compounds, cystine and uric acid, can be easily recognized by the great number and the position of the sharp maxima in the infrared spectrum of renal calculi.

The infrared spectra of all calculi were carefully searched for the presence of absorption maxima which could have been associated with the presence of organic matrix substance. The failure in finding a spectrum of such a substance is understandable considering the small amounts and the poor chemical characterization in which these substances can be expected.



Summarizing the attempts of a qualitative analysis of renal calculi by infrared spectroscopy the following considerations may be appropriate. In the infrared range of 2 to 15 microns the main interest concentrates on the "internal" vibrations of polyatomic ions. These are essentially due to vibrations of atoms relative to a central mass which may be considered at rest. In the case of the  $\text{PO}_4$  or  $\text{CO}_3$  -ions the central P or C - atom is at rest and the O-atoms vibrate relative to the central atoms. These vibrations are responsible for the broad maxima observed. They may be modified by other atoms or ions in the lattice as in the case of magnesium ammonium phosphate. The "lattice" vibrations in which the metal ion vibrates in relation to the complex anion cannot be observed in the spectral range used in these experiments. Under these conditions the infrared spectrum furnishes information essentially about the anions present in stones, modified to some extent by the cations. This information combined with the knowledge already available from X-ray studies of the lattice arrangement of anions and cations in calculi is sufficient to evaluate the spectrum effectively.

Compared with all the other methods used in stone analysis the infrared analysis furnishes a permanent record in a very short time. No special training or skill is necessary to handle the infrared spectrophotometer. It is an ideal method for analyzing small fractions of calculi in a short time. To the extent to which other clinical applications of the infrared method will be worked out more instruments will be available in clinical laboratories to meet the need of the urologist in routine analysis.

In the near future the instrument will also furnish reliable quantitative information on the composition of solid materials. In a new procedure the stones or parts of them, will be ground to a fine powder. A known quantity of this powder will be mixed with solid potassium bromide and pressed to form a platelet. The infrared spectrum of this platelet will give quantitative data for the composition of stones. The information gained in the above described mulling technique is only semi-quantitative. However, it will in many cases already satisfy the needs of the urologist.

The author is indebted to Drs. A. J. Butt and R. H. Leonard for the stone specimens and their interest, and to W. H. Harold for technical assistance.

#### BIBLIOGRAPHY

1. Daasch, L.W., and Smith, D. C., Analytical Chemistry 23, 853-868 (1951)
2. Hunt, J. M. and Turner, D. S., Analytical Chemistry 25, 1169-1174 (1953)
3. Hunt, J. M., Wisherd, M. P. and Bonham, L. C., Analytical Chemistry 22, 1478-1497 (1950)
4. Keller, W. D., Spotts, J. H., and Biggs, D. L., Am. J. of Science 250, 453-471 (1952)
5. Miller, F. A., and Wilkins, C. H., Analytical Chemistry 24, 1253-1294 (1952)
6. Panel discussion on urolithiasis, Urological Survey 4, 2-65 (1954)
7. Prien, E. L., and Frondel, C., J. Urology 57, 949-994 (1947)
8. Randall, H. M., Fowler, R. G., Fuson, N., and Dangel, J. R., Infrared Determination of Organic Structures, Van Nostrand Company, New York, 1952.
9. Schmidt, N. Ann. 27, 240.

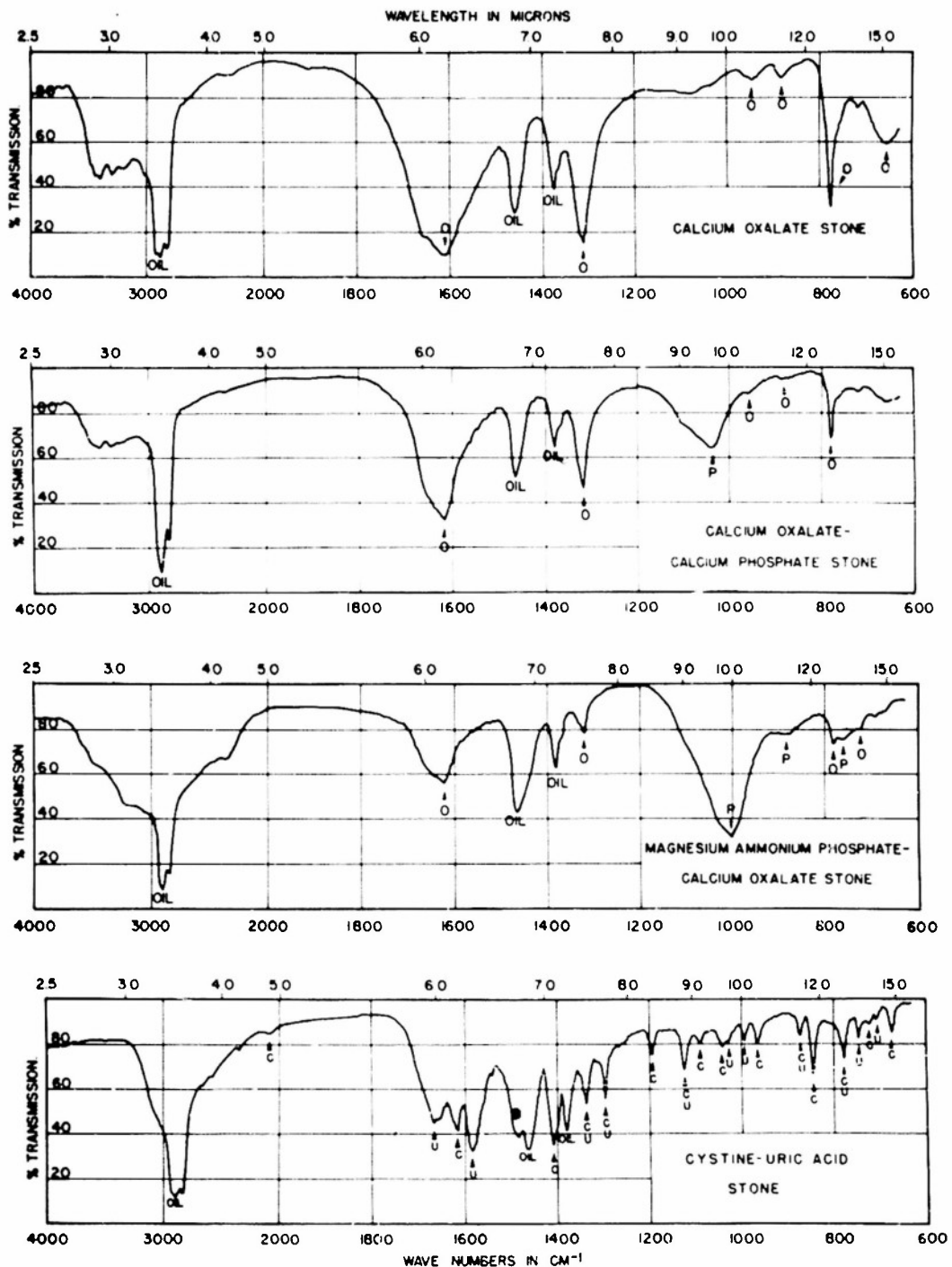


Figure 1.

Comparison of infrared spectra of four renal calculi.

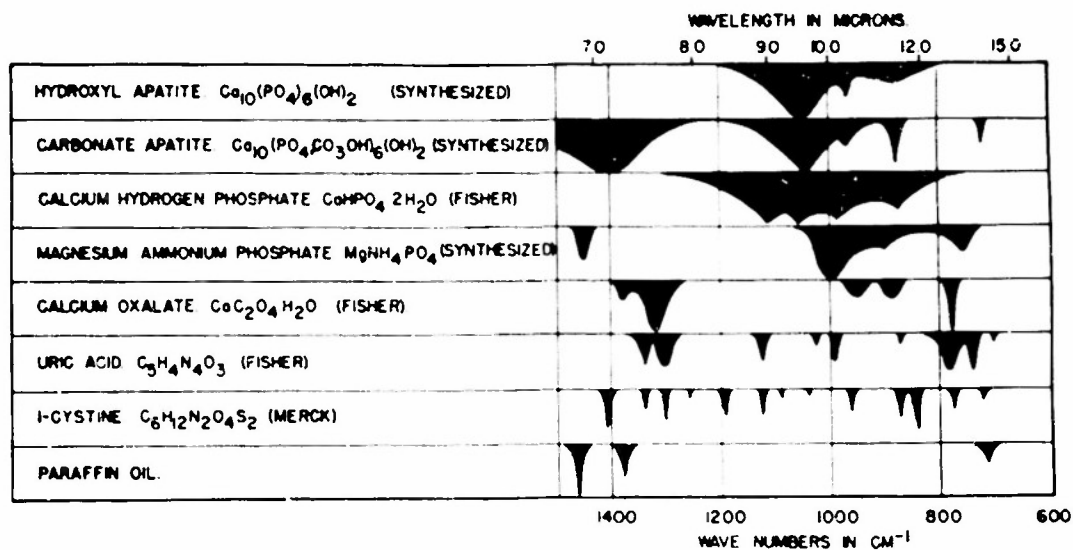


Figure 2.

Identification template with the long wave length region of infrared spectra of major components of renal calculi.

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